

# Toxicity of Diatomaceous Earth to Red Flour Beetles and Confused Flour Beetles (Coleoptera: Tenebrionidae): Effects of Temperature and Relative Humidity

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**ABSTRACT** Red flour beetles, *Tribolium castaneum* (Herbst), and confused flour beetles, *Tribolium confusum* (DuVal), were exposed for 8–72 h to diatomaceous earth (Protect-It) at 22, 27, and 32°C and 40, 57, and 75% RH (9 combinations). Insects were exposed to the diatomaceous earth at 0.5 mg/cm<sup>2</sup> on filter paper inside plastic petri dishes. After exposure, beetles were held for 1 wk without food at the same conditions at which they were exposed. Mortality of both species after initial exposure was lowest at 22°C but increased as temperature and exposure interval increased, and within each temperature decreased as humidity increased. With 2 exceptions, all confused flour beetles were still alive after they were exposed at 22°C, 57 and 75% RH. Mortality of both species after they were held for 1 wk was greater than initial mortality for nearly all exposure intervals at each temperature-humidity combination, indicating delayed toxic effects from exposure to diatomaceous earth. For both species, the relationship between mortality and exposure interval for initial and 1-wk mortality was described by linear, nonlinear, quadratic, and sigmoidal regression. Mortality of confused flour beetles was lower than mortality of red flour beetles exposed for the same time intervals for 46.7% of the total comparisons at the various temperature-relative humidity combinations.

**KEY WORDS** *Tribolium castaneum*, *Tribolium confusum*, flour beetles, diatomaceous earth, insecticides, surface treatment

INERT DUST FORMULATIONS of diatomaceous earth are fossilized skeletons of freshwater or marine diatoms that kill insects by abrading the cuticle and causing water loss through dessication (Korunic 1998). They are considered to be natural products, used as feed additives, and exempt from tolerance requirements. Although diatomaceous earth has been marketed for insect control in stored grains in the United States for >40 yr, older formulations were not very effective, and the high application rates often caused problems with the movement of treated grain (Korunic 1998, Subramanyam et al. 1998).

In recent years, several new formulations of diatomaceous earth have been registered to control insect pests in stored grains (Quarles 1992, Quarles and Winn 1996), and reviews of research have been published by Golob (1997) and Korunic (1998). The newer formulations are more effective than the older products and can be used at lower application rates. However, diatomaceous earth can potentially decrease the bulk density and flow rate of grain (Jackson and Webley 1994, Korunic et al. 1996). These effects on physical properties are largely dose dependent (Korunic 1997).

There are a number of factors that affect the insecticidal efficacy of diatomaceous earth formulations applied to stored grains. Formulations of diatomaceous earth from different sources show variation in

toxicity (Golob 1997), and variations in physical characteristics can affect product efficacy (Korunic 1997, 1998). Stored-product insects show variation in their susceptibility to diatomaceous earth (Carlson and Ball 1962, Desmarchelier and Dines 1987, Korunic 1998, Subramanyam et al. 1998). The toxicity of most diatomaceous earth formulations generally decreases as grain moisture content increases (Aldryhim 1990, 1993); however, temperature effects are often inconsistent and can be dependent on insect species and exposure conditions (Korunic 1998).

Most of the published studies with the new formulations of diatomaceous earth have involved various trials with stored grains, and there has been comparatively little work done on the efficacy of diatomaceous earth formulations applied as a surface treatment to control insect pests in flour mills, food processing plants, and storage warehouses. Loschiavo (1988a, 1998b) and White and Loschiavo (1989) conducted studies in which the merchant grain beetle, *Oryzaephilus mercator* (Fauvel), and the confused flour beetle, *Tribolium confusum* (DuVal), were exposed on filter papers treated with silica aerogel, a synthetic inert dust, for different time intervals. Toxicity increased with exposure interval but product efficacy was reduced when the beetles were provided with food after they were exposed.

Protect-It is a relatively new formulation of marine diatomaceous earth registered by Hedley Technologies, ON, Canada, for use on raw grains and on flooring surfaces. There are several published reports that indicate this product can control insect pests in stored grain (Korunic 1996, 1998). It also has been used in combination with heat treatments inside flour mills (Fields et al. 1997). The objectives of this test were as follows: (1) to determine exposure intervals required to kill red flour beetles, *Tribolium castaneum* Herbst, and confused flour beetles, 2 common beetle pests of indoor storage facilities; (2) to determine if beetles would subsequently die even if they were still alive after they were exposed; and (3) to determine effects of temperature and relative humidity on beetle mortality.

### Materials and Methods

Exposure studies were conducted at 3 temperatures (22, 27, or 32°C) and at 3 relative humidities (40, 57, or 75%) for a total of 9 temperature-humidity combinations. Humidity chambers were created by placing a waffle-type grid in the bottom of a 26.0 by 36.5 by 15-cm plastic box, and filling the box to the level of the grid with 1 of 3 saturated salt solutions,  $K_2CO_3$ , NaBr, or NaCl, which gave relative humidities of 40, 57, and 75%, respectively (Greenspan 1977). A sample of dry formulation of Protect-It was obtained from Hedley Technologies and stored in the laboratory at ambient conditions. The label rate for this product when used as a flooring surface treatment is 5 g/m<sup>2</sup>. Beetles were exposed in standard 100 by 15-mm plastic petri dishes with an internal diameter of 8.9 cm and area of 62 cm<sup>2</sup>; therefore, 31 mg of dust was used to treat each dish at the equivalent label rate.

Separate tests were conducted at each temperature-relative humidity combination in random order by using red flour beetles and confused flour beetles obtained from pesticide-susceptible colonies maintained at 27°C and 60% RH. For each species, each of 30 dishes was prepared by lining it with filter paper, weighing out 31 mg of Protect-It on an electronic balance, placing the dust on the filter paper, then using a metal probe to distribute the dust inside the dish. Ten adults (1–2 wk old) were placed in each dish, the dishes were covered with the lid, and all 60 dishes were put in a single humidity chamber containing 1 of the 3 salt solutions set inside 1 of the 3 incubators. Beetles were exposed for 8, 16, 24, 48, or 72 h, and there were 5 replicates plus an untreated control for each exposure interval. Upon completion of the exposure interval the dishes containing the beetles were removed from humidity chamber, beetles were classified as live or dead (initial mortality), transferred to new petri dishes lined with clean filter paper, and returned to the chamber. After an additional week the beetles were removed, classified as live or dead (1-wk mortality), and discarded.

Temperature and relative humidity inside the plastic boxes were monitored using HOBO data recorders (Onset Computer, Pocasset, MA). The test was ana-

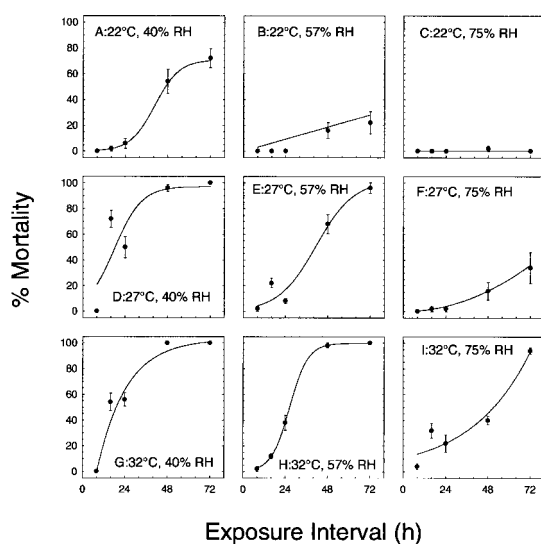


Fig. 1. Mortality of red flour beetles (mean  $\pm$  SEM) exposed for 8–72 h at 22, 27, and 32°C; 40, 57, and 75% RH on concrete treated with the Protect-It formulation of diatomaceous earth at the rate of 0.5 mg/cm<sup>2</sup>. Curve-fit lines are from equations in Table 1.

lyzed using the general linear models procedure (SAS Institute 1987), with beetle mortality as the response variable and species, temperature, relative humidity, and exposure interval as main effects. Initial mortality and 1-wk mortality were repeated measures. Control mortality was rare for both species and no corrections were necessary. Lack of fit tests (Draper and Smith 1981) were conducted using Table curve 2D software (Jandel Scientific 1996) to determine the amount of variation that could be explained by any model fit to the data (maximum  $R^2$ ), and the amount of variation explained by the given equation ( $R^2$ ), and to fit the appropriate regression curves to the data. The Kruskal-Wallis test, NPARIWAY procedure (SAS Institute 1987) was used to determine significant differences between mortality of red flour beetle versus confused flour beetle.

### Results

**General Statistical Analysis.** The main effects species ( $F = 492.1$ ;  $df = 1, 720$ ) temperature ( $F = 1192.7$ ;  $df = 2, 720$ ), relative humidity ( $F = 729.2$ ;  $df = 2, 720$ ), and exposure interval ( $F = 872.7$ ;  $df = 4, 720$ ), and the repeated measure initial versus 1-wk mortality ( $F = 92.5$ ;  $df = 1, 169$ ) were all significant ( $P < 0.01$ ). The temperature  $\times$  relative humidity ( $F = 25.9$ ;  $df = 4, 120$ ), the temperature  $\times$  exposure interval ( $F = 24.2$ ;  $df = 8, 720$ ), and the relative humidity  $\times$  exposure interval ( $F = 24.4$ ;  $df = 8, 720$ ) interactions also were significant ( $P < 0.01$ ).

**Red Flour Beetle.** Red flour beetle mortality after initial exposure increased as temperature increased and decreased as relative humidity increased (Fig. 1). At 22°C, mortality did not exceed 30% except for bee-

**Table 1.** Equations describing mortality of red flour beetle exposed for 8–72 h to diatomaceous earth (Protect-It) at the rate of 0.5 mg/cm<sup>2</sup>

Temp, °C	RH	Equation <sup>a</sup> parameters ± SE			R <sup>2</sup>	Max R <sup>2</sup>	% of max
		a	b	c			
22	40	72.7 ± 5.9	40.7 ± 3.1	6.9 ± 2.1 <sup>b</sup>	0.88	0.88	100
	57	—	0.40 ± 0.09 <sup>c</sup>	—	0.82	0.83	98.8
27	40	97.3 ± 7.5	17.9 ± 2.6	7.7 ± 2.9 <sup>b</sup>	0.71	0.93	76.3
	57	102.0 ± 8.2	41.3 ± 3.3	10.7 ± 1.9 <sup>b</sup>	0.91	0.95	95.8
	75	—	0.007 ± 0.001 <sup>d</sup>	—	0.51	0.51	100
32	40	103.0 ± 5.1	167.8 ± 16.0	0.06 ± 0.01 <sup>e</sup>	0.92	0.96	95.8
	57	79.9 ± 5.9	35.3 ± 4.3	5.0 ± 1.70 <sup>b</sup>	0.90	0.90	100
	75	10.8 ± 2.2	-33.4 ± 3.6 <sup>f</sup>	—	0.85	0.92	92.4

Beetles were exposed and held at 22, 27, and 32°C and 40, 57, and 75% RH. For all equations  $Y = \% \text{ mortality}$ ,  $x = \text{exposure interval in hours}$ .

<sup>a</sup> Regression for 22°C and 75% RH was nonsignificant ( $P \geq 0.05$ ), mortality =  $0.4 \pm 0.4\%$ .

<sup>b</sup> Sigmoid equations,  $y = a/[1 + \exp(-[x-b/c])]$ .

<sup>c</sup> Linear equation,  $y = ax + b$ , intercept not significantly different ( $P \geq 0.05$ ) from zero.

<sup>d</sup> Quadratic equation,  $y = a + bx^2$ , intercept not significantly different ( $P \geq 0.05$ ) from zero.

<sup>e</sup> Three-parameter nonlinear equation  $y = a - be^{(-cx)}$ .

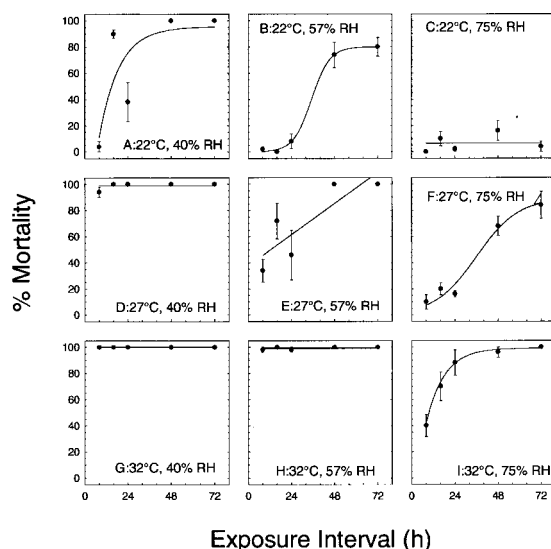
<sup>f</sup> Two-parameter nonlinear equation  $y = ae^{(-x/b)}$ .

tles exposed at 40% RH for 48 and 72 h (Fig. 1 A–C). Mortality of beetles exposed for 48 h at 27°C was  $96 \pm 2.4$ ,  $68 \pm 7.3$ , and  $16 \pm 6.8\%$  as humidity increased to 40, 57, and 75% RH, respectively (Fig. 1 D–F). At 32°C, mortality of beetles exposed for 48 h was  $100 \pm 0.0$ ,  $98 \pm 2.0$ , and  $40 \pm 3.2\%$  at 40, 57, and 75% RH, respectively (Fig. 1 G–I). Mortality generally increased as exposure interval increased, and within each temperature mortality was usually lower at 75% than at either 40 or 57% RH. The relationship between mortality immediately after exposure and the exposure interval for each temperature–humidity combination was described by sigmoidal, nonlinear, quadratic, and linear regression; and as relative humidity increased, the regression lines became more linear because mortality was approaching zero at the lower exposure intervals and decreasing at the higher intervals (Fig. 1; Table 1). Regression was not significant ( $P \geq 0.05$ ,  $y = \text{mean}$ ) for 22°C, 75% RH.

Mortality of red flour beetles after they were held for 1 wk at the same conditions was greater than initial mortality after exposure for nearly all intervals at each temperature–humidity combination (Fig. 2), indicating that the beetles continued to be affected by exposure to diatomaceous earth even after they were removed from the exposure arenas. At 22 and 27°C, mortality generally increased as temperature and the original exposure interval increased (Fig. 2 A–F); however, more beetles died when exposed for 16 h at 27°C, 40% RH (Fig. 2A) and at 27°C, 57% RH (Fig. 2E) than at 24 h. Fewer beetles died when exposed and held at 75% RH compared with 40 or 57% RH, and mortality at 5 of these exposure intervals was progressively lower as humidity increased. Nearly all beetles were dead 1 wk after being exposed for 8 and 16 h at 32°C, 40 and 57% RH, and held for 1 wk (Fig. 2 G and H); however, mortality of beetles exposed for 8 and 16 h at 75% RH was only  $40.8 \pm 8.4$  and  $70.0 \pm 11.0\%$ , respectively. The relationship between mortality 1 wk after exposure and holding at the same exposure conditions was described by sigmoidal, nonlinear, and linear regression (Fig. 2; Table 2). Regressions were

not significant ( $P \geq 0.05$ ,  $y = \text{mean}$ ) for 22°C, 75% RH; 27°C, 40% RH; or 32°C, 40 and 57% RH.

**Confused Flour Beetle.** Mortality of confused flour beetle exposed to diatomaceous earth also increased as temperature increased and decreased as relative humidity increased (Fig. 3). With 2 exceptions, all of the confused flour beetles were still alive after they were exposed for 8–72 h at 22°C, 57 and 75% RH (Fig. 3 B and C). As temperature increased to 27°C, mortality of beetles exposed for 24, 48, and 72 h began to increase, but nearly all were still alive after being exposed for 8 and 16 h (Fig. 3 D and G). Mortality of beetles exposed at 75% RH did not exceed 20% at any exposure interval (Fig. 3F), and was lower at 75% RH than at either 40 or 57% RH. Even at 32°C, mortality



**Fig. 2.** Mortality of red flour beetles (mean ± SEM) 1 wk after being removed from exposure and held at the same temperature and humidity conditions at which they were exposed. The independent variable ( $x$ ) is the original exposure interval. Curve-fit lines are from equations in Table 2.

**Table 2.** Equations describing mortality of red flour beetles 1 wk after they were removed from the exposure arenas and held for 1 wk at the same temperature (22, 27, and 32°C) and relative humidity (40, 57, and 75% RH) at which they were exposed

Temp, °C	RH	Equation <sup>a</sup> parameters ± SE			R <sup>2</sup>	Max R <sup>2</sup>	% of max
		a	b	c			
22	40	95.4 ± 10.4	173.6 ± 64.1	0.09 ± 0.04 <sup>b</sup>	0.55	0.88	62.5
	57	79.9 ± 5.9	35.3 ± 4.3	5.1 ± 1.7 <sup>c</sup>	0.89	0.89	100
27	57	37.3 ± 9.7	1.0 ± 0.2 <sup>d</sup>		0.43	0.59	72.9
	75	89.9 ± 10.0	36.0 ± 5.1	11.7 ± 3.0 <sup>c</sup>	0.82	0.84	100
32	75	99.1 ± 5.8	127.0 ± 38.1	0.09 ± 0.03 <sup>b</sup>	0.66	0.66	100

The independent variable (x) is the exposure interval. For all equations Y = % mortality, x = exposure interval in hours.

<sup>a</sup> Regressions for 22°C, 40% RH, 27°C, 40% RH, and 32°C, 40 and 57% RH were nonsignificant ( $P \geq 0.05$ ), mortality =  $6.4 \pm 2.2$ ,  $98.8 \pm 0.9$ ,  $100 \pm 0.0$ , and  $99.2 \pm 0.06\%$ , respectively.

<sup>b</sup> Three-parameter nonlinear equation  $y = a - be^{(-cx)}$ .

<sup>c</sup> Sigmoid equations  $y = a/[1 + \exp(-[x-b]/c)]$ .

<sup>d</sup> Linear equation,  $y = ax + b$ .

of beetles exposed for 8–24 h did not exceed 20%. The relationship between mortality immediately after exposure and the exposure interval for each temperature-humidity combination was described by quadratic and sigmoidal regression, and as humidity increased the regression lines became more linear because mortality was decreasing (Fig 3; Table 3). Regression was not significant ( $P \geq 0.05$ ,  $y = \text{mean}$ ) for 22°C, 75% RH.

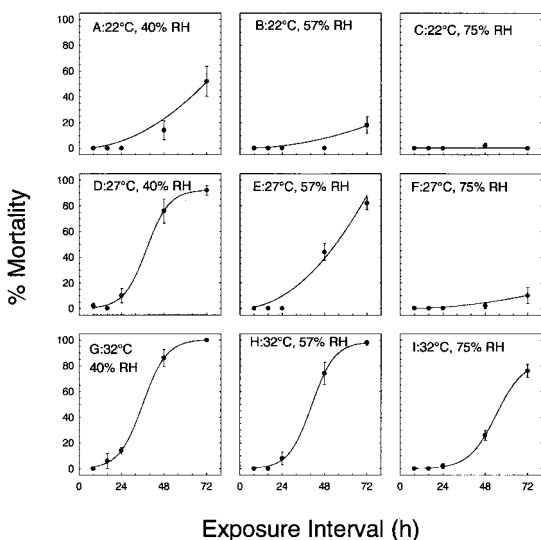
Mortality of confused flour beetles also was greater after the 1-wk holding period than after the initial exposures (Fig. 4) except for 22°C, 75% RH (Fig. 4C). Mortality at 27°, 40 and 57% RH increased as the initial exposure interval increased, with 100% mortality of beetles exposed for 72 h (Fig. 4d and E); however, mortality of beetles exposed and held at 75% RH was only  $54.0 \pm 12.9\%$  (Fig. 4F). All beetles were dead 1 wk after exposure for 24 h at 32°C, 40% RH (Fig. 4G),

but at 57 and 75% RH, 48- and 72-h exposures, respectively, were required to give 100% mortality (Fig. 4 H and I). Fewer beetles died when exposed and held at 75% RH versus 40 or 57% RH. The relationship between mortality immediately after exposure and the exposure interval for each temperature-humidity combination was described by sigmoidal, linear, and nonlinear regression (Fig. 4; Table 4). Regression was not significant ( $P \geq 0.05$ ,  $y = \text{mean}$ ) for 22°C, 75% RH.

Comparing the means for mortality after the initial exposure of red flour beetles in Fig. 1 and confused flour beetles in Fig. 3 indicated that mortality of the confused flour beetle after the initial exposures was less than mortality of the red flour beetle for 2 of 15, 8 of 15, and 11 of 15 comparisons at 22, 27, and 32°C, respectively (Table 5). This difference appeared to increase with temperature. Comparing the means for mortality after the 1-wk holding period of red flour beetles in Fig. 2 versus confused flour beetles in Fig. 4 shows that mortality of confused flour beetles was less than mortality of red flour beetles for 6 of 15, 7 of 15, and 7 of 15 comparisons. In the remainder of the comparisons, there was no significant difference between the 2 species, as shown in Table 5.

## Discussion

Results of this study show that mortality of red flour beetles and confused flour beetles exposed to the Protect-It formulation of diatomaceous earth at the rate of 31 mg/62 cm<sup>2</sup> (0.5 g/m<sup>2</sup>) was directly related to the exposure interval, the temperature and relative humidity at which the beetles were exposed, and species. As the exposure interval increased, mortality increased; furthermore, beetles exposed to diatomaceous earth subsequently died after they were removed from the treated environment. Other studies reported increased mortality of stored-product beetles exposed to inert dusts for increasing time intervals and subsequent mortality after the beetles were removed from exposure and held for additional time periods (Vrba et al. 1983, White and Loschiavo 1989, McLaughlin 1994). However, these studies were done only at 1 set of exposure conditions. White and Loschiavo (1989) exposed confused flour beetles for 6 h



**Fig. 3.** Mortality of confused flour beetles (mean ± SEM) exposed for 8–72 h at 22, 27, and 32°C; 40, 57, and 75% RH on concrete treated with the Protect-It formulation of diatomaceous earth at the rate of 0.5 mg/cm<sup>2</sup>. Curve-fit lines are from equations in Table 3.

**Table 3.** Equations describing mortality of confused flour beetle exposed for 8–72 h to diatomaceous earth (Protect-It) at the rate of 0.5 mg/cm<sup>2</sup>

Temp, °C	RH	Equation <sup>a</sup> parameters ± SE			R <sup>2</sup>	Max R <sup>2</sup>	% of max
		a	b	c			
22	40	—	0.01 ± 0.001 <sup>b</sup>	—	0.71	0.73	97.2
	57	—	0.003 ± 0.0007 <sup>b</sup>	—	0.49	0.59	83.1
27	40	92.4 ± 5.3	38.1 ± 2.5	6.3 ± 1.2 <sup>c</sup>	0.93	0.94	98.9
	57	—	0.02 ± 0.001 <sup>b</sup>	—	0.93	0.95	97.9
32	75	—	0.002 ± 0.001 <sup>b</sup>	—	0.28	0.29	96.5
	40	100.5 ± 4.2	36.1 ± 1.9	6.7 ± 0.9 <sup>c</sup>	0.92	0.96	95.8
	57	98.9 ± 4.7	40.8 ± 1.8	6.5 ± 1.2 <sup>c</sup>	0.95	0.95	100
	75	83.5 ± 15.0	54.1 ± 5.5	7.7 ± 4.1 <sup>c</sup>	0.96	0.96	100

Beetles were exposed and held at 22, 27, and 32°C and 40, 57, and 75% RH. For all equations Y = % mortality, x = exposure interval in hours.

<sup>a</sup> Regression for 22°C and 75% RH was nonsignificant ( $P \geq 0.05$ ), mortality =  $0.4 \pm 0.4\%$ .

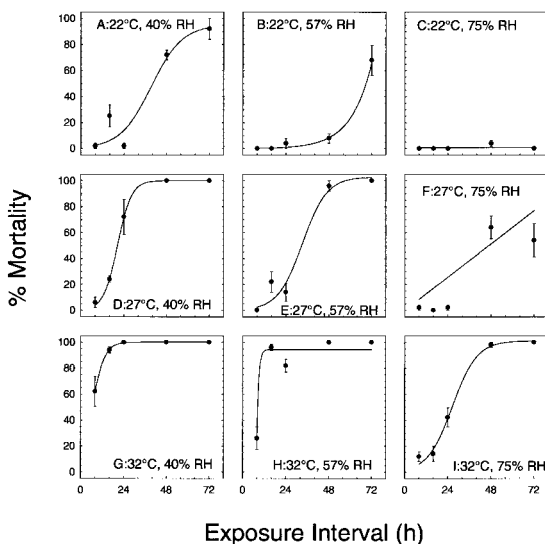
<sup>b</sup> Quadratic equation  $y = a + bx^2$ , intercept not significantly different ( $P \geq 0.05$ ) from zero.

<sup>c</sup> Sigmoid equations  $y = a/[1 + \exp(-[x-b]/c)]$ .

on paper treated with silica aerogel at the rate of 0.72 mg/cm<sup>2</sup>. Exposure and holding conditions were 25°C, 50% RH, and all beetles were dead after 3 d. McLaughlin (1994) exposed granary weevils, *Sitophilus granarius* (L.), and rice weevils, *Sitophilus oryzae* (L.), for 30 h at 25°C, 56% RH in aluminum pans treated with several diatomaceous earth products at the rate of 2 g/m<sup>2</sup>, and assessed mortality for 6 d after the weevils were removed from the diatomaceous earth. Final mortality was dependent upon the specific product. This apparent delayed toxic effect has not been noted in research publications in which *Tribolium* spp. were exposed to the newer formulations of diatomaceous earth.

There have been few studies regarding the effect of temperature on the susceptibility of stored-product beetles to diatomaceous earth, except for studies in which beetles were exposed on treated grains. In tests with amorphous silica, the granary weevil was more susceptible to silica dust at 30 versus 20°C, but the reverse was true for the confused flour beetle (Aldryhim 1990). Korunic (1998) stated that increased temperature increases the efficacy of diatomaceous earth for most beetle species, but not the red flour beetle and the confused flour beetle. However, the results of my test in which both *Tribolium* species were exposed directly to diatomaceous earth at controlled temperatures showed a progressive increase in mortality as temperature increased from 22 to 27 and 32°C. The beetles could have been more active at the higher temperatures, and picked up more of the diatomaceous earth particles as they moved about the exposure arena. Respiration and water loss also could have increased at higher temperatures. This positive increase in toxicity with temperature is similar to data reported for exposure studies with organophosphate insecticides (Turnbull and Harris 1986).

Most of the published research evaluating the effect of relative humidity on susceptibility of *Tribolium* spp. to inert dusts also has involved exposures on treated grain (Golob 1997, Korunic 1998). Water loss caused by exposure to diatomaceous earth would be expected to decrease with increasing relative humidity, thereby resulting in decreased mortality. LePatourel (1986) showed a 6- to 8-fold increase in the LC<sub>50</sub> of red flour beetles when they were exposed for 1 wk on wheat treated with silica dust and held at moisture contents of 11 compared with 16% (equivalent to ≈40–85% RH). Similar decreases in toxicity were reported when confused flour beetle were exposed on wheat treated with silica dust and held at increasing relative humidity (Aldryhim 1990). Although concentration was not a dosage factor in this study, the negative effect of relative humidity on the efficacy of Protect-It was evident by the increased exposure intervals required to give the same level of control of both *Tribolium* spp. exposed at 75% compared with 40 and 57% RH. The



**Fig. 4.** Mortality of confused flour beetles (mean ± SEM) 1 wk after being removed from exposure and held at the same temperature and humidity conditions at which they were exposed. The independent variable (x) is the original exposure interval. Curve-fit lines are from equations in Table 4.

**Table 4.** Equations describing mortality of confused flour beetles 1 wk after they were removed from the exposure arenas and held for 1 wk at the same temperature (22, 27, and 32°C) and relative humidity (40, 57, and 75% RH) at which they were exposed

Temp, °C	RH	Equation <sup>a</sup> parameters ± SE				Max R <sup>2</sup>	% of max
		a	b	c	R <sup>2</sup>		
22	40	95.1 ± 8.8	39.2 ± 3.7	8.7 ± 2.1 <sup>b</sup>	0.85	0.91	93.4
	57	0.14 ± 0.02	-11.6 ± 3.4 <sup>c</sup>	—	0.83	0.83	100
27	40	100.1 ± 4.3	20.4 ± 1.0	3.9 ± 0.8 <sup>b</sup>	0.90	0.91	99.0
	57	102.9 ± 6.2	33.1 ± 3.0	3.8 ± 1.6 <sup>b</sup>	0.92	0.95	96.8
	75	—	1.1 ± 0.02 <sup>d</sup>	—	0.62	0.80	77.5
32	40	100.2 ± 3.3	6.3 ± 1.1	3.5 ± 1.6 <sup>b</sup>	0.66	0.66	100
	57	94.5 ± 2.6	204210 ± 17624 <sup>e</sup>	—	0.85	0.90	92.3
	75	101.1 ± 4.0	26.6 ± 1.8	6.8 ± 1.5 <sup>b</sup>	0.94	0.94	100

The independent variable (x) is the exposure interval. For all equations Y = % mortality, x = exposure interval in hours.

<sup>a</sup>Regression for 22°C and 75% RH was nonsignificant ( $P \geq 0.05$ ), mortality =  $0.8 \pm 0.5\%$ .

<sup>b</sup>Sigmoid equations  $y = a/[1 + \exp(-[x-b]/c)]$ .

<sup>c</sup>Two-parameter nonlinear equation  $y = ae^{(-x/b)}$ .

<sup>d</sup>Linear equation  $y = a + bx$ , intercept not significantly different ( $P \geq 0.05$ ) from zero.

<sup>e</sup>Two-parameter nonlinear equation  $y = a - be^{-x}$ .

actual time interval in which *Tribolium* spp. are exposed on a treated commodity or treated surface should be considered a dosage factor, in addition to the actual application rate (Barson 1991).

In general, *Tribolium* spp. appear to be less susceptible to inert dusts and diatomaceous earths than other stored-product beetles (Korunic 1998). In my study, mortality of confused flour beetles was significantly lower than mortality of red flour beetles for 46.7% of the total comparisons for the various time intervals, which indicates that the confused flour beetle may be less susceptible to Protect-It than the red flour beetle. In studies with conventional insecticides, the order of susceptibility between these 2 species is apparently dependent on the specific insecticide and formulation (Arthur 1998a, 1998b); therefore, the relative suscep-

tibility of the red flour beetle and the confused flour beetle could be different with other formulations of diatomaceous earth.

Both the red flour beetle and the confused flour beetle can infest indoor storage facilities and processing plants. If these facilities are not climate controlled, the environmental differences between seasons could affect the efficacy of diatomaceous earth products such as Protect-It when they are used to control *Tribolium* spp. When internal relative humidity is high, as would occur during the summer, product efficacy would be reduced, and treatment areas may have to be enlarged to reduce the possibility of the beetles escaping from the treated environment. Also, because the confused flour beetle may be less susceptible to Protect-It than the red flour beetle, species identi-

**Table 5.** Mortality of red flour beetles versus mortality of confused flour beetles after the initial (means ± SEM compared for Fig. 1 versus 3) exposures and 1-wk exposures (means ± SEM compared for Fig. 2 versus 4)

Temp, °C	% RH	Exposure	Exposure interval, h				
			8	16	24	48	72
22	40	Initial	NS	NS	NS	*	NS
		1 wk	NS	*	*	**	NS
	57	Initial	NS	NS	NS	*	NS
		1 wk	NS	NS	NS	**	NS
	75	Initial	NS	NS	NS	NS	NS
		1 wk	NS	*	NS	NS	NS
27	40	Initial	NS	**	**	*	*
		1 wk	**	**	**	NS	NS
	57	Initial	NS	**	*	*	*
		1 wk	**	*	NS	NS	NS
	75	Initial	NS	NS	NS	NS	NS
		1 wk	NS	**	**	NS	NS
32	40	Initial	NS	**	**	**	NS
		1 wk	*	*	NS	NS	NS
	57	Initial	NS	**	**	**	NS
		1 wk	*	NS	**	NS	NS
	75	Initial	**	**	*	*	**
		1 wk	**	**	**	NS	NS

Differences between species at each temperature, relative humidity, and exposure interval determined by the Kruskal-Wallis test under the NPARIWAY procedure of SAS (SAS Institute 1987). NS, no significant difference; \* and \*\* indicate that mortality of confused flour beetle < mortality of red flour beetle, at  $P < 0.05$  and  $P < 0.01$ , respectively.

cation is important because the specific control strategies may have to be directed to control the least susceptible of the 2 species.

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This article reports the results of research only. Mention of a proprietary chemical or a trade name does not constitute a recommendation or endorsement by the USDA for its use.

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